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Alterations in Nerve and Muscle Compound Action Potentials After Acute Acrylamide Administration

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The early deficits of neurotoxicity induced by acrylamide were examined in rats by comparing nerve and muscle action potentials before and 24 hr after exposure to acrylamide (25, 50 or 100 mg/kg). No changes were seen in the nerve action potential amplitude or duration. The 25 mg/kg dose produced a more variable nerve conduction velocity. There was also a significant broadening of the muscle compound action potential. Neither of these effects were seen in the fasted controls. However, the lengthening of the relative refractory period of the muscle action potential was highly correlated with losses in body weight in the treatment groups and was identical to changes seen in control animals which were fasted for 24 hr. The slowed conduction of the muscle action potential may be a precursor of the nerve terminal damage which results from chronic exposure. Changes in the muscle refractory period, on the other hand, appear to be secondary to the loss in body weight which accompanies acrylamide administration.

Introduction

Chronic exposure to acrylamide results in progressive signs of neurotoxicity which have been described in both morphologic (*1*) and behavioral terms (*2*). The effects on various components of peripheral nerves have been the most extensively studied aspects of acrylamide toxicity. There is a selective loss of the distal portions of both motor (*3*) and sensory nerves (*4*, *5*), with the large diameter fibers affected before smaller axons (*6*).

Clinically, acrylamide toxicity is characterized by paresthesia (*1*) and muscle weakness (*2*). Although these effects are related to the neuropathy, it is important to note that behavioral deficits have been reported prior to the appearance of histologic nerve damage. Some behavioral changes occur as a result of an effect on membrane excitability. However, the electrophysiologic characteristics of peripheral nerve and skeletal muscle are attenuated by acrylamide only after long periods of chronic dosing, an effect which correlates more closely with the destruction of nerve axons (*7–9*) than with behavioral deficits.

Unfortunately, the effects of acrylamide on electrophysiologic parameters have usually been examined using indirect, noninvasive techniques. Inaccuracy in these measurements probably masked any subtle effects of the drug on axonal and muscle excitability. With more accurate techniques it might be possible to demonstrate that the muscle weakness and paresthesia following acrylamide administration are linked to a loss of electrophysiologic responsiveness in the nerve and/or muscle, and that both effects precede the histologic damage which occurs only after long-term administration of acrylamide.

The purpose of this study was to examine whether changes could be detected in the electrophysiologic activity of peripheral nerve and muscle using direct measurements after relatively short-term exposure to acrylamide.

Methods

To reduce the variability in measurements, each animal in these experiments served as its own

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control. The control parameters of nerve and muscle activity were recorded from the left hindlimb. One week later the animal was dosed with acrylamide. Twenty-four hours after acrylamide administration, another set of parameters was recorded from the contralateral hindlimb.

Male Sprague-Dawley rats (190-240) were anesthetized with 50 mg/kg pentobarbital, adminstered intraperitoneally. The left hindlimb was dissected to expose the sciatic nerve and triceps surae muscles. Pairs of platinum-iridium stimulating and recording electrodes were placed on the sciatic nerve for obtaining control measurements from the nerve. The muscle electrophysiologic activity was also recorded differentially. Following the data collection the hindlimb was sutured and the animal was allowed to recover from anesthesia. Care was taken not to sever or damage any of the major hindlimb innervation during these procedures and cursory observation of the animal after recovery from anesthesia revealed no detectable deficit in locomotion as a result of these procedures. One week was allowed for the animal to recover from the effects of the surgery.

Groups of five animals from which control measurements had been taken were then dosed with either 25, 50 or 100 mg/kg of acrylamide or 1 ml saline intraperitoneally. At 24 hr after administration, each animal was again anesthetized with 50 mg/kg pentobarbital and the right hindlimb was dissected. The right sciatic nerve and triceps surae muscles were recorded in a similar manner to the left.

Following this set of electrophysiologic recordings, the sciatic nerves from both limbs were fixed *in situ* in 10% formalin for histologic examination. After staining with hemotoxylin-eosin the nerves were cut in both longitudinal and cross section and mounted. The coded slides were evaluated by an unbiased morphologist for evidence of structural abnormalities.

Based on pilot studies, the acrylamide treated animals were expected to lose a significant amount of weight. In order to account for effects which might be caused by a nonspecific and acute weight loss, control animals were fasted for 24 hr before recording from the contralateral hindlimb.

The procedure for recording the electrophysiologic parameters before and after acrylamide has been described previously (*10*). Supramaximal rectangular pulses (0.02 msec duration) were applied to the sciatic nerve at 15- sec intervals. The nerve and muscle action potentials were recorded from 100 consecutive stimuli. Each response was converted to its digital equivalent, analyzed and stored by our microprocessor system. The muscle action potential latency was measured as the time in milliseconds from the stimulus artifact to the onset of muscle depolarization. The nerve conduction velocity was calculated from the length of nerve between the stimulating and recording electrodes and the conduction time. For both the nerve and muscle action potentials, duration was measured from onset of depolarization to return to the baseline; peak amplitude was calculated in millivolts.

Twin pulse stimuli were used to determine the refractory period of the muscle action potential. As the stimulus interval was varied between 3.5 and 20 msec, the two muscle action potentials were recorded and analyzed. The relative refractory period was taken as an exponential function (*11*) of the second action potential relative to the first. From these functions, the time at which the second potential reached 75% of its maximal amplitude was calculated and was taken as a standardized measure of refractoriness for comparison between treated and control groups.

Changes in each parameter of the action potentials between the left and right hindlimb were analyzed as a function of dose by analysis of covariance. In addition, the refractory period measurements were analyzed by the sign test. Changes which differed at $p < 0.05$ were judged to be statistically significant.

Results

The effects of acrylamide on the nerve and muscle action potentials are summarized in Table 1. The only significant change in nerve activity was an increased variability in conduction velocity after 25 mg/kg. The other characteristics of the wave form at this dose and all characteristics at the 50 and 100 mg/kg does were unaffected by acrylamide.

Alterations in the muscle action potential after acrylamide administration can be seen qualitatively as a change in the shape of the wave form. One such example is shown in Figure 1. Although there was no significant change in the conduction velocity which was calculated from the onset of the action potential depolarization, there was a marked broadening of the wave form. This was confirmed statistically as an increase in the duration of the muscle action potential as indicated in Table 1 and was seen after all three doses of acrylamide.

Acrylamide also induced a prolongation and increased variability of the relative refractory period of the muscle action potential. The stimulus interval required for 75% recovery of the action potential in each treatment group is compared in Table 2. With the contralateral untreated side being used as

Table 1. Effects of acrylamide and fasting on nerve and muscle electrophysiologic activity.

	Control, unfasted	Control, fasted	Acrylamide 25 mg/kg	Acrylamide 50 mg/kg	Acrylamide 100 mg/kg
Nerve action potential					
Conduction velocity, m/sec	31.7 ± 1.4	35.8 ± 3.2	79.0 ± 24.9[a]	38.0 ± 3.2	34.0 ± 3.5
Duration, msec	1.05 ± 0.09	0.83 ± 0.03	1.02 ± 0.13	0.94 ± 0.29	0.85 ± 0.35
Amplitude, mV	0.49 ± 0.07	0.99 ± 3.11	0.42 ± 0.15	0.72 ± 0.10	0.92 ± 0.10
Muscle action potential					
Latency, msec	1.87 ± 0.06	2.06 ± 0.21	1.81 ± 0.11	1.79 ± 0.22	1.76 ± 0.23
Duration, msec	1.96 ± 0.13	1.98 ± 0.11	2.58 ± 0.33[b]	2.42 ± 0.25[b]	2.48 ± 0.26[b]
Amplitude, mV	0.58 ± 0.05	0.68 ± 0.20	0.51 ± 0.09	0.58 ± 0.15	0.81 ± 0.20

[a] $p < 0.001$, compared with unfasted control.
[b] $p < 0.005$, compared with unfasted control.

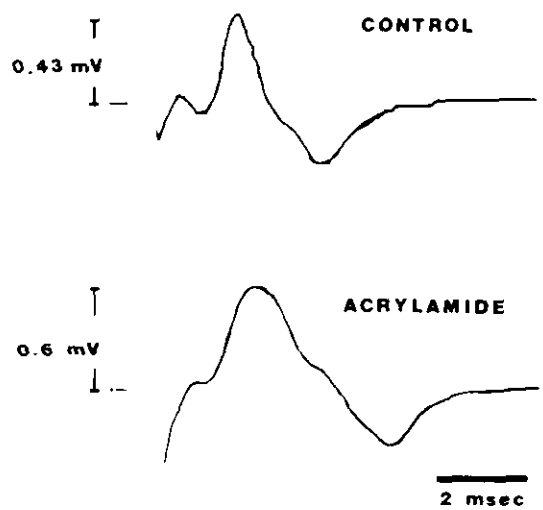

FIGURE 1. Effect of acrylamide on the muscle compound action potential. Each panel shows the computer average of 100 consecutive action potentials: (top) control response of one animal; (bottom) response from the contralateral limb of the same animal 24 hr after exposure to 50 mg/kg acrylamide.

the control, acrylamide (100 mg/kg) significantly increased the refractory period from 5.25 to 8.85 msec. Interestingly, control rats which were fasted for 24 hr also showed a marked prolongation in the relative refractory period which was indistinguishable from the rats receiving 100 mg/kg acrylamide. Concurrently with this prolongation, the refractory period was more variable among the treated animals than in the same animals prior to treatment. Although the increased variability only reached statistical significance for the fasted animals and the 100 mg/kg acrylamide-treated animals, the standard errors are clearly larger in all treated groups, compared to the unfasted controls.

As we had seen previously in pilot studies, acrylamide treatment resulted in a loss of body weight. A significant loss of approximately 19 grams was seen in the animals receiving 100 mg/kg acrylamide and in the control rats which were fasted for 24 hr. This is shown in Figure 2. The loss in body weight for all groups was correlated statistically with the prolongation in the muscle action potential relative refractory period (correlation coefficient $r = 0.96$).

There are several incidental observations which should also be noted. The acrylamide treated animals, especially those receiving 50 or 100 mg/kg, showed signs of behavioral toxicity after 24 hr of exposure. Although these were not quantitated, the animals appeared to be hyperreflexic, especially to nonspecific somatosensory stimulation. They also resisted being handled. Resting tremor was noted in several animals receiving 100 mg/kg but locomo-

Table 2. Effect of acrylamide and fasting on the relative refractory period of the muscle action potential.

	75% Refractory period, msec (mean ± SEM)	% of unfasted control	Sign test[a]
Control, unfasted	5.25 ± 0.51	100	
Acrylamide			
25 mg/kg	7.4 ± 2.70	114	NS
50 mg/kg	6.4 ± 1.20	120	NS
100 mg/kg	8.85 ± 2.79	184	$\alpha < 0.01$
Control, fasted	8.82 ± 2.87	200	$\alpha < 0.01$

[a] Compared with unfasted control.

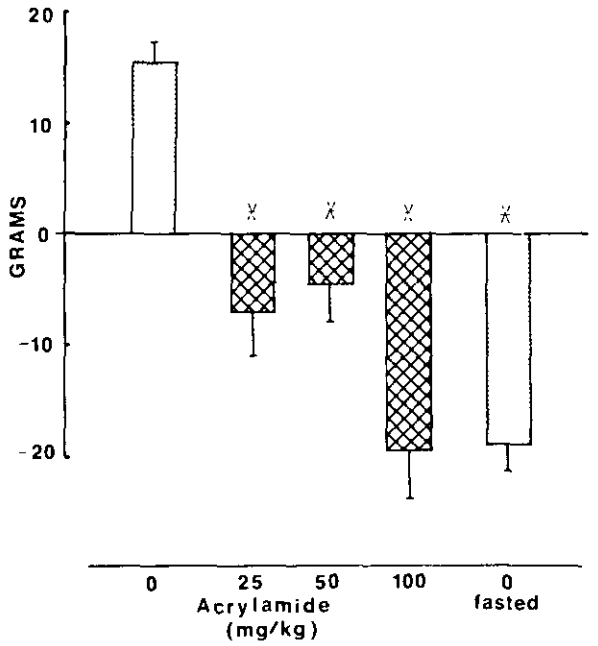

FIGURE 2. Effect of acrylamide on body weight. Each bar is the mean ± SEM of the weight lost or gained in each group during the 24 hr treatment period. The cross-hatched bars show the acrylamide-treated groups and the stippled bars show the control groups. The control group on the right was fasted for 24 hr. Asterisks (*) indicate $p < 0.05$ when compared to the untreated control.

tion was not markedly altered in any of the treated animals.

No sign of peripheral neuropathy was noted in the light microscope slides of either hindlimb nerve at any of the three acrylamide doses. In some cases, however, there was evidence of increased vascularization and an inflammatory response on the left side, which was the first operated side. The sciatic nerves from the right hindlimb, which were fixed immediately following the electrophysiologic recording, were normal.

Discussion

These results show that alterations in nerve and muscle function can be detected after only 24 hr exposure to acrylamide. Although an accurate correlation was not attempted between the electrophysiologic effects and changes in the animals' behavior, it was noted that the animals receiving 50 or 100 mg/kg also showed behavioral signs of acrylamide intoxication (tremor, hyperreflexia, etc.).

It is important to note that the changes in the muscle action potential were more pronounced than in the nerve and suggests that the initial effects of acrylamide are on muscle function rather than nerve. This would be consistent with the observations of Tilson and Cabe (*2*), who have reported that hindlimb muscular weakness is the earliest sign of acrylamide intoxication. It is interesting to note that Leswing and Ribelin (*8*) reported a selective prolongation of conduction distally through nerve terminals and muscles of cats and monkeys after acrylamide administration. Since the neuromuscular junction is affected only after longer periods of exposure to acrylamide (*12*), our observations along with others suggest that the "dying back" of the axons actually begins with a change in muscle. The loss then proceeds to the motor nerve terminals which are affected before motor axons (*3*).

Only one change was noted in the sciatic nerve, an increased variability in the conduction velocity after 25 mg/kg. This effect is difficult to interpret since larger doses had no effect. It may be, however, that the low dose produced a transient excitatory response which is unrelated to the subsequent peripheral neuropathy. Several other neurotoxic agents have been shown to be biphasic in their effects (*13*), producing an initial hyperexcitability and then a more pronounced and prolonged depressant effect. Only the latter, incidently, has been correlated with structural damage to the nervous system (*13*).

It is important to note that both these changes—in nerve and muscle—were demonstrated after only 24 hr exposure to acrylamide. The functional electrophysiologic deficit appeared to correlate with our uncontrolled observations of behavioral changes in the high dosed animals. However, these functional deficits occurred prior to any structural pathology in the nerve. Only one study, using behavioral techniques (*2*), has shown motor dysfunction with an earlier onset (12 hr after exposure to 200 mg/kg). However, no mention was made in this case of weight loss, which would be expected to be great, and one can question the contribution of generalized weakness to the result.

In our study, the above-mentioned changes are significant because they did not occur in control animals which had been fasted to lose comparable weight over the same time period. This is contrasted by the effects on the muscle refractory period in which there was an obvious correlation with weight loss. This increased refractoriness of the muscle may contribute to the gross weakness observed in animals after short-term dosing but does not preclude other factors (e.g. neuropathy) from contributing to the motor dysfunction seen after longer term exposure. In any case, the more important effect is the slowed muscle conduction caused by acrylamide.

Others have achieved a separation of muscle dysfunction and weight loss (2), but only after very low doses and at least one week of chronic exposure. In view of this, the electrophysiologic changes after 24 hr are all the more striking. One can speculate that these early, subtle changes in the muscle, and perhaps the nerve, are the first indication that a progressive distal-to-proximal neuromuscular dysfunction has begun, and if allowed to continue will result in structural damage.

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